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Rous–Whipple Award

The Rous–Whipple Award was established by the American Society for Investigative Pathology to recognize a career of outstanding scientific contribution.

The 1992 recipient of the Rous–Whipple Award, Dr. Russell Ross, delivered a lecture entitled "Atherosclerosis: A Defense Mechanism Gone Awry" after the presentation of the award on Tuesday, April 7, 1992 in Anaheim, California, at the meeting of the Federation of American Societies for Experimental Biology.

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Rous-Whipple Award Lecture

Atherosclerosis: A Defense Mechanism Gone Awry

Russell Ross

Atherosclerosis, the principal source of cerebral and myocardial infarction, gangrene of the extremities, and loss of function of organs and/or tissues, causes the majority of deaths in the United States, Europe, and Japan.[1] Throughout the years, this disease has been called a degenerative process, a proliferative process akin to a benign tumor, and an accumulation of lipid within the artery wall similar to the accumulation of sludge within a pipe. Data accumulated in numerous laboratories worldwide have provided evidence to support a hypothesis postulated by von Rokitansky[2] and Virchow[3] and later modified by French[4] that led to the development of the response-to-injury hypothesis originally formulated in 1973[5] and tested and further modified by Ross.[6–9] This hypothesis suggests that the lesions of atherosclerosis represent a specialized form of a protective, inflammatory-fibroproliferative response to various forms of insult to the artery wall. Depending upon the nature and duration of the insult, the protective response may become excessive and over many years *in its excess become a disease process.*

The most recent modification of the response-to-injury hypothesis[9] suggests that the numerous, different forms of insult to the endothelium and to the cells of the artery wall begin at specific sites in the arterial tree with a chronic, inflammatory response, featuring peripheral blood monocytes and T lymphocytes, which adhere to the endothelium and invade the artery wall. At these sites, many of the monocytes become converted to macrophages. During activation, they express a series of different genes, including genes for cytokines and growth regulatory molecules, and they, and possibly some of the T cells that accompany them, may undergo replication within the artery wall. As a consequence, various growth factors and cytokines are released by these activated leukocytes, some of which may result in smooth muscle migration and proliferation within the intima of the artery. These events culminate in the lesions that represent different stages of this specialized, inflammatory, fibroproliferative response and that contain varying amounts of associated lipid and lipoprotein. Our understanding of the process of atherogenesis results from research into the complex cellular and molecular interactions associated with lesion initiation and progression in both experimental animals and, recently, in humans.

The advanced, fibroproliferative lesions of atherosclerosis, the fibrous plaques, or advanced, complicated lesions, take one of two forms. In common atherosclerosis, they usually present as an asymmetric thickening of the intima, or innermost layer of the artery (Figure 1). This is the type of lesion that most frequently leads to clinical sequelae. The second type of advanced lesion is associated with immune rejection, such as may occur in some cardiac transplants, and tends to be more symmetric or concentric (Figure 2). In either case, the enlarging lesion may or may not be associated with compensatory dilation of the artery. The absence of such dilation could lead to a decrease in lumen diameter and ultimately to clinical sequelae.

Accumulating data in humans and experimental animals suggest that, if the sources of the insult, eg, hypercholesterolemia, continue, then the initial lesions of atherosclerosis will progress with time to become advanced, occlusive lesions. In some instances, these lesions may lie dormant, or even regress and disappear. The growth regulatory mol-

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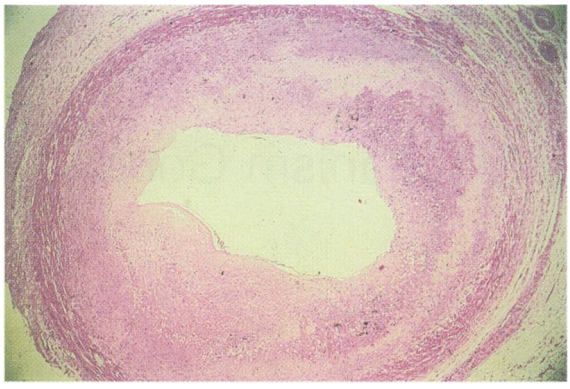

Figure 1. *A light micrograph of a human left circumflex coronary artery, which contains an advanced lesion of atherosclerosis (a fibrous plaque), is shown. This advanced lesion is characterized by a dense cap of connective tissue that overlies a region, which is looser in construction, containing macrophages, intra- and extracellular lipid, and cell debris. The media beneath this region is thinned. This is a characteristic appearance of a fibrous plaque, which in this case occludes approximately 50% of the artery.*

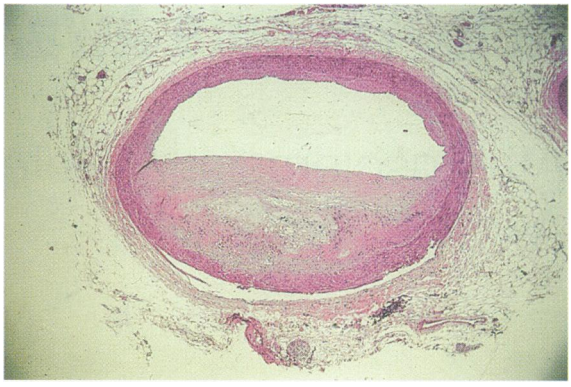

Figure 2. *This light micrograph shows a portion of a left, anterior, descending coronary artery obtained from a heart transplant rejected within approximately 6 months posttransplant. The lesion in this coronary artery is typical of that associated with graft rejection atherosclerosis. As can be seen, it is a circumferential lesion, in contrast to the lesion in Figure 1. The cellular makeup of the two lesions is similar to the degree that there is a dense band of smooth muscle cells intermixed with macrophages and connective tissue around the perilumenal region. Deeper, beneath this band of smooth muscle cells, is a region relatively richer in macrophages and T lymphocytes. Both the lesion from common atherosclerosis (Figure 1) and the one depicted here contain an abundance of smooth muscle macrophages and T cells. However, the response in immune rejection is characteristically concentric.*

ecules and cytokines that seem to be important in these processes can be derived from all of the cells involved, including the macrophages, possibly the T lymphocytes, the endothelium lining the artery, as well as the endothelium lining the vasa vasorum in the lesions, and the smooth muscle cells. The genes expressed by the different cells within the lesions play critical roles in determining whether the lesions will regress or progress. As these molecules and their roles are better understood, it should be possible to interrupt their activities, to reverse them, and possibly prevent the major complications of coronary artery, cerebrovascular, and peripheral vascular disease. Recent studies provide promising evidence for approaches to prevent lesion initiation and progression, as well as induce lesion regression.

The Response-to-Injury Hypothesis of Atherogenesis

The response-to-injury hypothesis proposes that various, possibly different forms of insult may develop between the lining endothelium and the underlying cells of the artery wall. In hyperlipidemic individuals, they seem to be due principally to the lipids and lipoproteins associated with hyperlipidemia,[5–9] whereas they may also may result from molecules yet to be identified with cigarette smoking, hypertension, diabetes, or possibly even some infectious agents. Diabetes is often complicated by varying forms of hyperlipidemia; thus, lipids may be critical, associative factors of atherogenesis in this disease. In any case, each of the risk factors seems to be associated with the generation of biologically active agents that may lead to dysfunctional changes in the endothelium. These changes alter the normal physiological characteristics of the endothelium, resulting in the ubiquitous, specialized, inflammatory response associated with atherogenesis.

The normal, functional characteristics of the endothelium include its capacity to act as 1) a permeability barrier; 2) a nonthrombogenic, nonleukocyte-adherent surface; 3) a source of vasoactive (vasoconstrictive and vasodilative) molecules; 4) a source of growth regulatory molecules; and 5) a source of connective tissue matrix macromolecules, among others. One or more of these normal, functional attributes of the endothelium may be altered by substances associated with the risk factors. For example, hyperlipidemic or hypercholesterolemic individuals often have elevated levels of low-density lipoprotein (LDL) and usually depressed levels of high-density lipoprotein.[10] Accumulating data suggest that in persons with elevated LDL levels the LDL may become oxidized, possibly during uptake and transcytosis by the endothelium.[11] By the time the LDL particles are deposited within the subendothelial space, many may have been modified or, more specifically, oxidized by the endothelial cells. Further oxidation may also result from interaction with macrophages and smooth muscle cells (to be discussed below). The modified lipoproteins, depending upon the level of oxidation, may have nu-

merous and possibly deleterious effects on the different cells of the artery wall. Not only can they act as chemoattractants for monocytes, but they can also have toxic effects upon the endothelium and the underlying smooth muscle cells. Such oxidized LDL (oxLDL) can be taken up by specialized receptors on the surface of the monocyte-derived macrophages and smooth muscle cells. Thus, lipid can accumulate in the macrophages through uptake by scavenger receptors and putative oxLDL receptors. With continued uptake of oxLDL, the macrophages become foam cells and establish the first, ubiquitous lesion of atherosclerosis, the fatty streak. Platelets may become involved at these sites, due to separations that occur between retracted endothelial cells, exposing the underlying cells or connective tissue matrix. This often occurs at branches and bifurcations where changes in the flow characteristics of the blood are common. Lipid-filled foam cells have been seen to emigrate from the lesion back into the bloodstream in hypercholesterolemic nonhuman primates.[12] The exposed foam cells or connective tissue matrix can become sites where aggregates of platelets and mural thrombi may tend to form. Thus, in the process of lesion formation and progression, foam cell-T cell-rich lesions (fatty streaks) can become converted, with time, to lesions enriched in smooth muscle cells. The smooth muscle cells in the lesions may be derived from cells that migrated from the underlying media into the intima, or the foam cell deposits may occur within preexisting accumulations of smooth muscle cells, called intimal cushions.

If the injurious substances continue to be exposed to the cells of the artery wall, the inflammatory response may continue and the lesions of atherosclerosis may expand until they impinge upon the flow of blood. During the process of lesion expansion, a fibroproliferative process takes place, which is the natural culmination of the inflammatory response that helped to initiate the lesions. The ultimate representation of the advanced lesion is the so-called fibrous plaque or advanced, complicated lesion, which contains a fibrous cap of dense, connective tissue containing embedded smooth muscle cells and monocyte-derived macrophages and T lymphocytes that covers a deeper repository of varying mixtures of macrophages, lipid, necrotic debris, smooth muscle cells, and loose connective tissue (Figure 3).

The nature and source of the dysfunctional changes in the endothelium that result from diabetes, hypertension, or cigarette smoking are still poorly understood. The changes in the artery probably represent variations on the theme described above. They culminate in the different lesions that represent the excessive, inflammatory smooth muscle, fibroproliferative response we call atherosclerosis.

The Lesions of Atherosclerosis

As indicated above, at least three types of lesions of atherosclerosis have been described: the fatty streak, the intermediate or fibrofatty lesion, and the advanced lesion termed a fibrous plaque or advanced, complicated lesion. A number of reports have described the intimal changes that seem to precede the formation of fatty streaks in animals and in humans. Simionescu et al[13,14] detected subtle changes by electron microscopy in lesion-prone areas of the aortic arch of rabbits in the first 2 weeks of diet-induced hypercholesterolemia, before the time that monocytes enter the artery and become foam cells. These changes are characterized by progressive accumulation of small unilamellar and multilamellar vesicles within the extracellular matrix, located in the space between the endothelium and the internal elastic lamina. The vesicles contain large amounts of unesterified cholesterol and are described as extracellular liposomes. They are thought to represent particles that some investigators have ascribed to cell debris or precipitated lipoprotein. Masuda and Ross[15,16] observed similar structures in the arterial intima of monkeys fed a relatively low-level hypercholesterolemic diet. Such structures have also been seen in human lesions. However, there are few data concerning the earliest changes that precede lesion formation in humans. Davies et al[17] have described early inflammatory events in human coronary arteries that are virtually identical to those described in monkeys and rabbits. In experimental animals and in humans, these lipid particles are ingested by the monocyte-derived macrophages and smooth muscle cells, both of which can become foam cells within the fatty streak. In humans, the fatty streak tends to be a mixture of lipid-filled macrophages, T lymphocytes, and lipid-containing smooth muscle cells.[18] In experimental animals lacking intimal cushions or preexisting collections of intimal smooth muscle cells, such as occurs in humans, the fatty streak tends to consist of lipid-filled, monocyte-derived macrophages with varying numbers of T lymphocytes.[15,19–21]

The intermediate lesion represents a stage during which replication of both macrophages and smooth muscle cells may occur, resulting in alternating layers of macrophages, T cells, and smooth muscle that

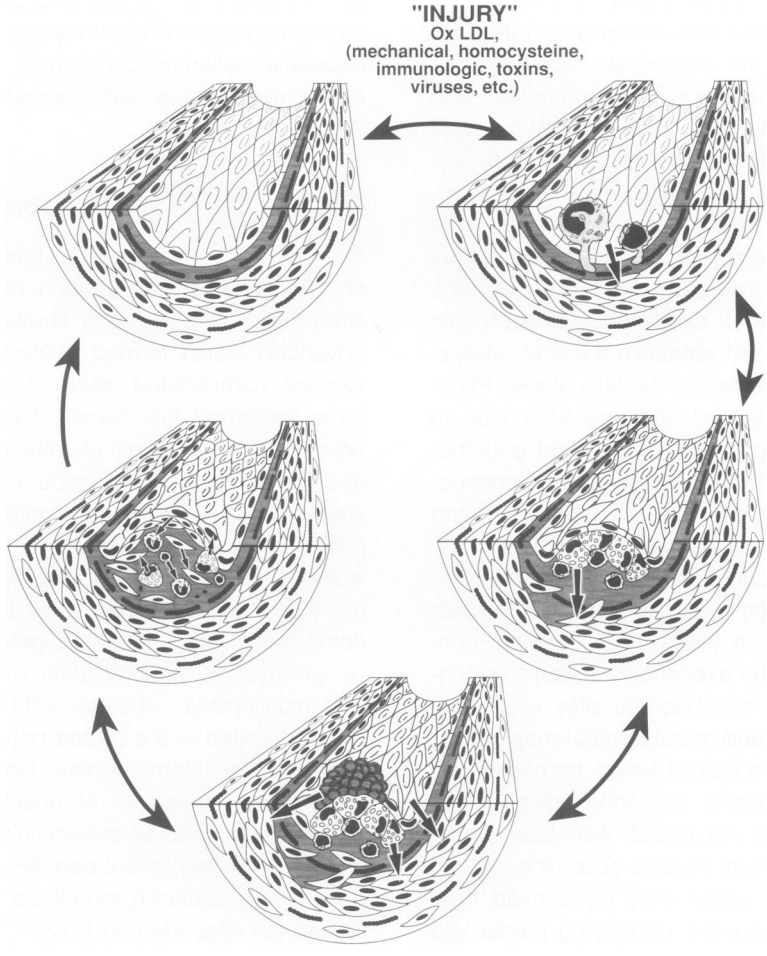

Figure 3. *The response-to-injury hypothesis of atherosclerosis is shown. Several different sources of injury to the endothelium can lead to endothelial cell dysfunction. One of the parameters associated with endothelial cell dysfunction that results from exposure to agents, such as oxLDL, is increased adherence of monocytes/macrophages and T lymphocytes. These cells then migrate between the endothelium and localize subendothelially. The macrophages become large foam cells because of lipid accumulation and, with the T cells and smooth muscle, form a fatty streak. The fatty streak can then progress to an intermediate, fibrofatty lesion and ultimately to a fibrous plaque. As the lesions accumulate more cells, and the macrophages scavenge the lipid, some of the lipid-laden macrophages may emigrate back into the bloodstream by pushing apart the endothelial cells. On doing so, those at sites such as branches and bifurcations, where the blood flow is irregular with eddy currents and back currents, may become thrombogenic sites that lead to formation of platelet mural thrombi. Such thrombi can release many potent growth regulatory molecules from the platelets that can join with those released by the activated macrophages and possibly by lesion smooth muscle cells into the artery wall. Platelet thrombi can also form at sites where endothelial dysjunction may have occurred. Ultimately, the formation and release of numerous growth regulatory molecules and cytokines form a network established between cells in the lesion, consisting of activated macrophages, smooth muscle, T cells, and endothelium, and lead to progression of the lesions of atherosclerosis to a fibrous plaque or advanced, complicated lesion. Each of the stages of lesion formation is potentially reversible. Thus, lesion regression can occur if the injurious agents are removed, or when protective factors intervene to reverse the inflammatory and fibroproliferative processes. Reproduced with permission from Nature.*

form varying amounts of connective tissue. With lesion progression, a fibrous cap forms over the lesion. Teleologically, this event may be an attempt to protect the artery wall and perhaps to provide some tensile strength to this potentially weakened site in the artery. With continued cell replication, necrosis, lipid accumulation, and connective tissue formation, the lesions increase in size until they become fibrous plaques with a dense fibrous cap overlying the central part of the lesion and a thinner fibrous cap at the shoulders of the lesion that contains numerous macrophages. Data from Davies and Thomas[22] suggest that events such as sudden death may occur due to weak spots in the shoulders of advanced, complicated lesions of atherosclerosis or at sites enriched in macrophages and relatively poor in connective tissue. At these parts of the lesion, depending upon the anatomic site (branches or bifurcations) where the lesion is located, the stresses and strains from blood flow may lead to cracks, fissures, or tears in the thinner parts of the fibrous cap that cover the shoulders of the lesion. Should such changes take place, hemorrhage may occur from the lumen into the lesion or from vasa vasora within the lesion itself, leading to thrombosis, which may occlude the artery and lead to sudden death.

Stary[18] examined the coronary arteries of individuals who died between birth at full term and 29 years of age and found that the earliest identifiable lesions of atherosclerosis consist of isolated groups of macrophage-derived foam cells. Approximately 45% of infants in the first 8 months of life have such fatty streaks, which seem to accumulate preferentially in areas of eccentric intimal thickening but lack any smooth muscle cells. He theorized that the generation of extracellular lipid is somehow involved in the progression of the fatty streaks to more advanced lesions. His observations agree with evidence reviewed by McGill[10] that in coronary arteries increasing surface involvement by fatty streaks precedes the formation of advanced lesions.

The role of mural thrombosis in lesion progression is not well understood, although it has been observed in every hyperlipidemic nonhuman primate (over 100 examined) in our studies of hypercholesterolemia-induced atherogenesis[12,15,16,19] and in the human coronary artery specimens examined by Davies et al.[17] Should mural thrombi be involved, numerous growth regulatory molecules and cytokines released from the activated platelets could play an important role in enhancing the processes of cell migration and proliferation, connective tissue formation, and lesion progression.

The Location of Atherosclerotic Lesions in Relation to Blood Flow

The clinical effects of atherosclerosis are mainly evident in medium-sized muscular arteries, particularly the coronary, carotid, basilar, vertebral, superficial femoral, and iliac arteries, as well as in the aorta, the principal elastic artery in the body. Data from image analysis studies of lesions in arteries of hypercholesterolemic minipigs[23] and a large population of humans aged 15 to 37 suggest that such lesions are most likely to develop at: a) the entrance regions of arteries, such as the ascending aorta, the origins of the iliac arteries, the anterior descending, and circumflex coronary arteries; b) the lateral leading edges of the flow divider at the principal branches from the aorta; and less often at c) areas not associated with a particular orifice. Regions immediately distal to the large branch points have a low probability of lesion development. Although a relationship with hemodynamic events is apparent, the localization of the lesions does not show a strong correlation with specific hemodynamic forces, such as high or low shear stress, and is more likely related to unusual forces, such as eddy currents and back flow.[24] Such alterations in hemodynamic or rheological forces, as well as in wall shear stress, may induce changes within the cytoskeletal apparatus of the endothelial cells. It has been suggested that these shape changes somehow lead to alterations in endothelial cell gene expression.

Cellular Components of Atherosclerotic Lesions

The use of cell-specific monoclonal antibodies has permitted an accurate delineation of the cellular composition of each of the different stages of lesion formation and progression.[25–30] Gown et al[21] described three forms of raised lesions in adults 34 to 58 years of age: fibrofatty lesions (equivalent to fatty streaks), fibrous plaques, and advanced plaques. Fibrofatty lesions consist almost entirely of macrophages and T lymphocytes with relatively few smooth muscle cells. In contrast, the fibrous plaques contain large numbers of smooth muscle cells and macrophages with variable numbers of T lymphocytes. Although smooth muscle proliferation had been considered the *sine qua non* of atherosclerosis, it is now clear that both smooth muscle and macrophage proliferation occur in the lesions in experimental animals[31] and in humans.[32] Using immunocytochemistry and cell-specific monoclonal antibodies together with antibodies to the nuclear cell cycle traverse-specific antigen, the proliferating cell nuclear antigen, it has been possible to demonstrate cycling smooth muscle and macrophages.[32] The various cellular interactions and molecules potentially responsible for the replication of these cells are discussed below.

Jonasson et al[33] examined biopsy specimens of lesions from internal carotid arteries of patients 57 to 75 years of age. They subdivided the lesions into four regions and mapped the distribution of cell types in each region. Macrophages constituted 60% of the cell population in the core of the lesion, whereas they made up 24% of the cells in the fibrous cap and 18% of the cells in the shoulder. T lymphocytes were most common in the shoulder (22%) and fibrous cap (18%) but were rare in the necrotic core (9%). No macrophages or lymphocytes were found in the intima or media of normal arterial tissue. Both $CD4^+$ and $CD8^+$ T cells were identified throughout the fibrous plaques. Few granulocytes, B lymphocytes, or natural killer cells were detected.

Specific Cellular Interactions

Because the lesions of atherosclerosis begin as a chronic, inflammatory process, the first events probably include leakage of plasma constituents into the artery wall due to increased permeability of the endothelium. The next response to injury consists of an early stage of inflammation in which there is increased adherence of leukocytes, specifically, peripheral blood monocytes and T lymphocytes, in clusters on the surface of the endothelium (Figure 4).[9,15,17,19,20,34–37] This increased leukocyte adherence is due to the formation of specific sets of adhesive, cell-surface glycoproteins on both the leukocytes and the endothelium that act in a ligand-receptor fashion.[38–43] The presence of several selectin and integrin classes of molecules, including intercellular adhesion molecule-1[39] and vascular cell adhesion molecule (athero-endothelial leukocyte adhesion molecule),[41] can lead to increased adherence of monocytes and lymphocytes to the endothelium. Chemotactic factors can be generated by the endothelium and perhaps by intimal cells, inducing the leukocytes to penetrate actively between the endothelial cells, separate the endothelial junctions, and directly migrate into the subendothelial space, where they localize within the intima (Figure 5).[15,19,44–52] Many of the monocytes become active as macrophages and in their role as scavenger cells attempt to remove deleterious molecules such as oxLDL. Thus, the initial cellular interactions seem to take place at the surface of the endothelium, between monocytes, T lymphocytes, and endothelium, and among monocyte-derived macrophages and the overlying endothelium, adjacent T lymphocytes, or neighboring smooth muscle cells.

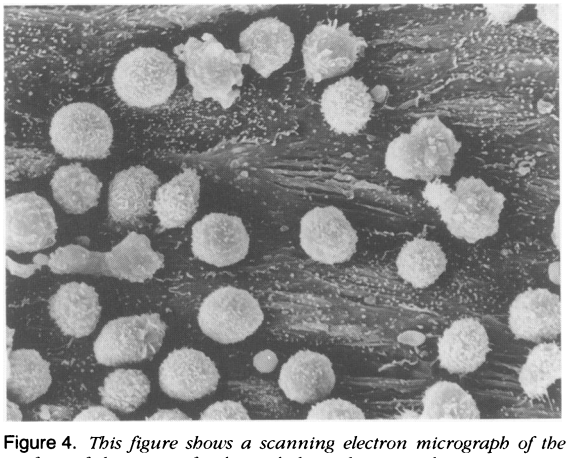

Figure 4. *This figure shows a scanning electron micrograph of the surface of the aorta of a hypercholesterolemic nonhuman primate. This monkey had been made hypercholesterolemic at levels of approximately 250 mg/dl about 1 month before sacrifice. Regions, such as those depicted in this micrograph, could be observed throughout the arterial tree, in which clusters of leukocytes adhere to the surface of the endothelium. This increased leukocyte adherence occurs after the cells start to roll along the surface, become sticky, and adhere due to the expression of a series of cell-surface, adhesive glycoproteins both on the leukocytes and on the endothelium. This is the first stage of the formation of the fatty streak.*

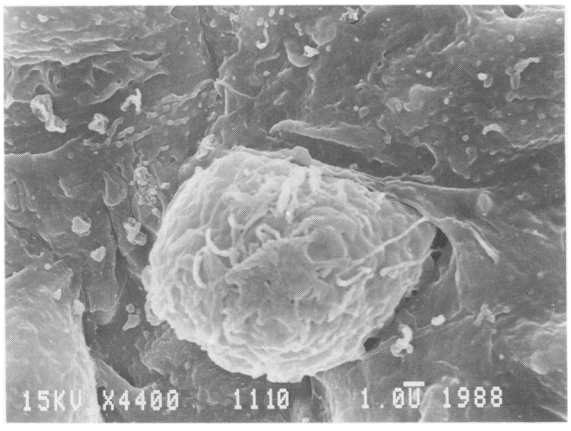

Figure 5. *This figure demonstrates a leukocyte that has been chemotactically attracted to enter into the artery wall and has already parted endothelial cells and inserted a process at the interendothelial junction. This process of chemotaxis can be generated by a number of molecules that can be expressed by endothelium and/or smooth muscle, including MCP-1, oxLDL, and IL-8. The continual entry into the artery wall of leukocytes and monocytes, such as this cell, leads to the development of the fatty streak, which results from conversion of monocytes to macrophages and ingestion of lipid and lipoproteins.*

Various genes can be expressed in each of these different cells, depending upon the nature of the stimuli and may result in the formation of cytokines and growth regulatory molecules. The nature of the genes expressed will determine whether macrophage replication, smooth muscle migration and replication, possibly T cell replication, and chemotaxis of additional monocytes from the blood into the lesion will occur. Such activation of the monocyte-derived macrophages could generate a host of growth regulatory molecules and cytokines (Figure 6), which could have profound effects on the neighboring cells. Similarly, gene expression and transcription in smooth muscle cells (Figure 7) could result in the formation of collagen, elastic fiber proteins, and proteoglycans, as well as growth regulatory molecules and cytokines that could have profound effects upon their neighbors. The endothelial cells and the T lymphocytes could also respond in a similar fashion. Thus, a complex network of cellular interactions may occur to promote progression from a macrophage-T cell-rich lesion to one that contains increasing numbers and amounts of smooth muscle cells and connective tissue, respectively. This fibroproliferative response is the hallmark of the fibrous plaque or advanced, complication lesion.

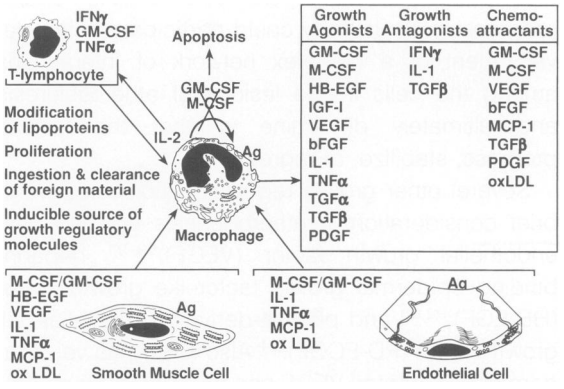

Figure 6. *The potential roles of the macrophage in atherogenesis. The reverse arrows between the T lymphocyte and the macrophage suggest that some form of immune response may occur during atherogenesis. Interactions between T cells and macrophages can result in proliferation of each of these cell types through IL-2 and CSFs, respectively. All of the cells with which the macrophages can interact, namely, T lymphocyte, smooth muscle, and endothelium, can present CSF to the macrophages to maintain cell viability and prevent apoptosis and cell death. The cells may participate in further macrophage activation and replication. In addition, smooth muscle and endothelium can present antigens at their surfaces and secrete chemoattractants for macrophages, including MCP-1 and oxidized LDL, as well as factors that can alter macrophage metabolism, such as IL-1 or TNF-α. When the macrophages are activated, they can produce an extraordinary number of biologically relevant molecules, some of which are listed in the diagram, in their capacity to induce or inhibit replication of endothelium, smooth muscle, or macrophages, and to make chemoattractants for each of these cell types. Reproduced with permission from Nature.*

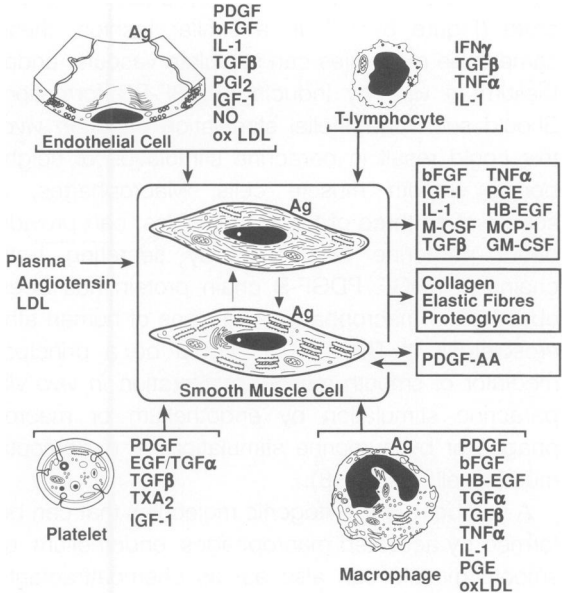

Figure 7. *The two potentially different phenotypic states of the smooth muscle cell. In the synthetic phenotype, it is presumed that smooth muscle cells can form connective tissue molecules as well as growth factors, such as PDGF-AA, and can stimulate themselves and their neighbors. In their interactions with the overlying endothelium and neighboring T lymphocytes, platelets, and macrophages, smooth muscle cells can respond to the different cytokines, growth regulatory molecules, and vasodilator and vasoconstrictor substances that can be generated from these cells, as well as substances from the plasma, such as angiotensin. Thus, the genes that are expressed in the different phenotypic states by the smooth muscle (listed to the right), as well as those expressed by the neighboring cells (listed next to each cell) in the artery wall that result from these cellular interactions will determine the outcome as to whether a lesion will progress or regress. Reproduced with permission from Nature.*

Diamond and Karnovsky[53] observed that in the kidney there are numerous mesangial cell proliferative changes that suggest analogies between glomerulosclerosis and the lesions of atherosclerosis. Among the analogies are: 1) similarities in morphology and function between the glomerular mesangial cell and the arterial smooth muscle cell; 2) the presence of activated macrophages in both lesions; 3) the presence of angiotensin receptors; 4) calcium-dependent, contractile responses to a number of mediators, including angiotensin, arginine vasopressin, and platelet-activating factor, and in the proliferative response; and 5) the presence of histochemically demonstrable platelet-derived growth factor (PDGF) in both sets of lesions.[54] These analogies may also hold for a number of other fibroproliferative responses associated with chronic inflammation in numerous organs and tissues, such as pulmonary fibrosis, rheumatoid arthritis, and wound repair.

Growth Regulatory Molecules and Cytokines

Although many growth regulatory molecules and cytokines may be formed within the lesions of atherosclerosis, a few may play dominant roles in this process. These include: both chains of PDGF (PDGF-A and PDGF-B),[55–58] two forms of colony-stimulating factor (CSF), M-CSF and GM-CSF,[59,60] basic fibroblast growth factor (bFGF),[61–64] insulin-like growth factor-I (IGF-I),[65,66] transforming growth factor-β (TGF-β),[67–78] and the cytokines interleukin-1 (IL-1)[79–83] and tumor necrosis factor-α (TNF-α).[84–88] PDGF, bFGF, and IGF-I may be critical to the proliferation of smooth muscle cells and, possibly, endothelium. The CSFs may play a role in macrophage stability and replication. TGF-β is perhaps the most potent stimulator of the synthesis of connective tissue matrix macromolecules, including various forms of collagen, proteoglycans, and elastic fiber proteins, and is also a potent inhibitor of the replication of many cells, including smooth muscle.

Although the cytokines IL-1 and TNF-α have numerous effects upon smooth muscle cells, they can also result in smooth muscle proliferation *in vitro*. They do so in a fashion similar to TGF-β by inducing PDGF-A chain gene expression in the smooth muscle cells and autocrine stimulation of the cells

by the PDGF-AA, which they synthesize and secrete (Figure 8).[77,78] In a similar fashion, these same three molecules can stimulate vascular endothelium *in vitro* by inducing PDGF-BB formation. Should such endothelial stimulation occur *in vivo*, this could result in paracrine stimulation of neighboring smooth muscle cells. Macrophages, a source of all three of these molecules, can provide direct paracrine stimulation by secreting both chains of PDGF. PDGF-B chain protein has been observed in macrophages in lesions of human atherosclerosis.[54] Thus, PDGF could be a principal mediator of smooth muscle proliferation *in vivo* via paracrine stimulation by endothelium or macrophages or by autocrine stimulation by the smooth muscle cells (Figure 8).

A number of the mitogenic molecules that can be formed by activated macrophages, endothelium, or smooth muscle can also act as chemoattractants for some of the other cells involved. For example, PDGF and IGF-I are potent chemoattractants for smooth muscle cells, FGF for endothelium, and M-CSF for monocyte-derived macrophages. As noted in Figures 5 and 6, macrophages and smooth muscle cells can also generate additional chemoattractants for monocytes, including monocyte chemotactic protein-1 (MCP-1) and oxLDL. Depending upon the factors induced in macrophages or smooth muscle, they could further enhance the inflammatory response and the accumulation of the different cell types within the lesions.

What has become clear from studies of atherogenesis in several animal models and in humans is that most of these molecules seem to be expressed and formed at some stage of lesion initiation and/or progression. Thus, they could participate in the development of a complex network of interactions among the cells in the lesions of atherosclerosis and ultimately determine whether the lesions progress, stabilize, or regress.

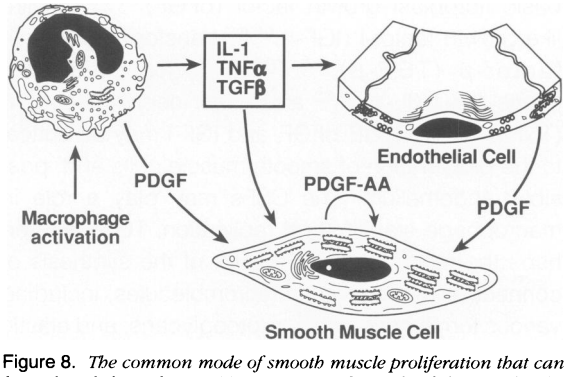

Figure 8. *The common mode of smooth muscle proliferation that can be induced through IL-1, TNF-α, or TGF-β. Each of these molecules can be formed by activated macrophages and, when they are exposed to endothelial or smooth muscle cells, each of these molecules will induce PDGF-B or -A chain gene expression, respectively. In in vitro studies, the mitogenic activity induced in endothelium or smooth muscle by IL-1, TNF-α, or TGF-β can be totally abolished by a neutralizing polyclonal antibody to PDGF. In addition to its capacity to form IL-1, TNF-α, and TGF-β, the macrophage can directly form PDGF and, thus, directly induce smooth muscle cell replication as well. Reproduced with permission from Nature.*

Several other growth regulatory molecules worth brief consideration in atherogenesis are: vascular endothelial growth factor (VEGF);[89–91] heparin-binding epidermal growth factor-like growth factor (HB-EGF);[92,93] and platelet-derived endothelial cell growth factor (PD-ECGF).[94] Also known as vascular permeability factor, VEGF can be formed by macrophages and smooth muscle cells, increases endothelial permeability, and could serve as a potent mitogen for endothelial cells, and thus could be angiogenic.[89–91] VEGF is a secreted protein with 18 to 20% homology with PDGF including conservation of all eight cysteine residues. Two classes of high-affinity (Kd 10^{-11} mol/L) binding sites have been identified for VEGF on endothelial cells. There is relatively little information concerning its presence or possible role in atherogenesis.

HB-EGF has a fairly extensive amino acid sequence homology with epidermal growth factor (EGF), particularly with conservation of specific cysteine residues within the molecule that retain the tertiary structure and presumably their capacity to bind to the EGF receptor.[92] Unlike PDGF, FGF, and HB-EGF, EGF is not a particularly potent mitogen for smooth muscle cells. There is little evidence that EGF plays any meaningful role in the process of atherosclerosis. Possibly an exception, HB-EGF is as potent a mitogen *in vitro* for smooth muscle as PDGF, and it can be synthesized by both macrophages and smooth muscle.[92,93] Although information on this molecule is increasing from *in vitro* studies, little is known of the role, if any, that it may play *in vivo*. Because of its potency as a smooth muscle mitogen, further work should determine whether or not it is involved in atherogenesis.

An angiogenic molecule, PD-ECGF is a 45-Kd single chain polypeptide that is both mitogenic and chemotactic for endothelial cells.[94] It was discovered as a source of angiogenic activity in platelets. Both PD-ECGF and its receptor have been isolated and purified. PD-ECGF is present in arterial smooth muscle cells as well as platelets, and it has been suggested that it may help to maintain the integrity of the overlying endothelium of arteries. Its role in atherogenesis, if any, remains to be determined.

In addition to the growth regulatory molecules and cytokines discussed above, several other small molecules can have profound effects upon endothelium

and smooth muscle and, in turn, upon atherogenesis. Two such molecules made by endothelium and smooth muscle, nitric oxide (NO)[95–99] and prostacyclin (PGI_2),[100] are potent, vasoactive agents that can induce arterial dilation and, at the same time, can actively prevent adhesion of platelets. NO is formed in endothelial cells from the terminal guanido nitrogen of L-arginine.[101] It can inhibit replication of smooth muscle cells *in vitro* and seems to be one of the principal agents responsible for maintaining vasomotor tone in the artery wall.[99,102] NO seems to act by opposing the effects of vasoconstriction due to agents such as phenylephrine, angiotensin, or thromboxane A_2.[99] NO also inhibits adhesion of leukocytes to endothelium as well as platelets. Its role as an antiatherogenic agent, although not proven, may turn out to be quite important.

Another set of small molecules, which may be relevant to atherogenesis, are the ecto-ADPases, which are aspirin-insensitive enzymes that metabolize adenosin triphosphate (ADP) from platelets and can act as antithrombotic agents. The release of such ecto-ADPases could affect thrombosis by degrading ADP from platelets, which would otherwise augment platelet aggregation.[103]

The Risk Factors

Hypercholesterolemia and oxLDL

Hypercholesterolemia has long been known to be associated with increased incidence of lesions of atherosclerosis and can induce these lesions, as described earlier from studies of nonhuman primates,[12,15,16,19] rabbits,[20,104] swine,[34,44] pigeons,[45] and hamsters.[105] Recognition of the possible role of oxLDL in atherogenesis came from the work of Carew et al[106] and Kita et al,[107] who observed that the antioxidant, probucol, causes a statistically significant diminution in the size of the lesions of atherosclerosis in the WHHL rabbit, in which hypercholesterolemia is due to a single gene defect resulting in deficient LDL receptors. This was the first direct *in vivo* evidence that oxidized forms of LDL might be important in atherogenesis. These and other groups observed that oxLDL is injurious to cells, including endothelial cells, which have the capacity to modify LDL as they transcytose the LDL from the plasma into the artery wall.[11,108–110] oxLDL is chemotactic for monocytes and can induce the formation of the chemoattractant MCP-1 in macrophages and endothelium.[51,52] oxLDL may participate in initiating monocyte transmigration between the endothelium into the artery wall. The formation of oxLDL also generates lysophosphatidylcholine,[52] which can act as a potent chemoattractant for human monocytes. oxLDL can be taken up by macrophages, depending upon the state of oxidation, either by the scavenger receptor or via other receptors, including a putative oxidized LDL receptor. Immunohistochemistry with monoclonal antibodies against oxLDL demonstrates its presence within macrophages in human lesions of atherosclerosis as well as in lesions in hypercholesterolemic animals.[111–116]

Until recently, the data for the effectiveness of antioxidant therapy reside in the studies with rabbits, as described above. Sasahara et al (manuscript submitted) have recently completed a study of the effects of probucol in nonhuman primates made hypercholesterolemic by diet. Their studies demonstrated for the first time that antioxidants are as effective in nonhuman primates as they are in rabbits in helping to reduce the lesions of atherosclerosis. The monkeys were hypercholesterolemic for 11 months and were on probucol or the carrier for the probucol for a period of 8 months. Quantitative analysis of intimal lesion size demonstrated a statistically significant reduction in lesions that correlated with the antioxidant activities of probucol and not with its cholesterol-lowering property. Thus, oxLDL seems to play a role in the process of atherogenesis in nonhuman primates and possibly in humans as well. It should be noted that oxLDL is not necessarily the only component in lipids that induces atherogenesis but may certainly be an important one. These observations raise the possibility of the use of other antioxidants, such as vitamin E,[117] β-carotene, and vitamin C, in the prevention of atherosclerosis.

Hypertension

The means by which hypertension induces lesions of atherosclerosis is poorly understood. Genetic studies of hypertension have used a restriction fragment length polymorphism for renin in the Dahl/JR salt-susceptible rat, which co-segregates with the existing hypertension, suggesting that a genetic locus linked with this polymorphism is somehow responsible for the hypertension. Renal dysfunction has been shown to be important in some forms of essential hypertension, demonstrable most clearly in black, hypertensive patients, demonstrating important differences in susceptibility to hypertension among different races and between genders.[118]

Both medial smooth muscle hypertrophy and intimal hyperplasia occur in animal models of experi-

mentally induced hypertension, as well as in arteries of humans with hypertension.[119] In the rat, these hypertrophic events have been shown to be due in part to an increase in ploidy and in the size of the medial smooth muscle cells. There seems to be an interrelationship between mechanisms that induce smooth muscle proliferation and smooth muscle contraction. Elements of the sympathetic nervous system, which are activated in hypertension, can induce vascular wall growth. Angiotensin-II is elevated in many hypertensives and can directly mediate smooth muscle hyperplasia. Phenylephrine has an autocrine growth-stimulating effect on the rat aorta, and smooth muscle cells from spontaneously hypertensive rats show an enhanced proliferative response to PDGF in culture when compared with those from normal rats. Other vasoactive substances such as serotonin, endothelin-1, and thrombin can also stimulate smooth muscle replication, whereas inhibitors of vasoconstriction, such as atrial natriuretic peptide may have an opposing effect.

Similar to changes observed in experimentally induced hypercholesterolemia, Chobanian et al[120] have demonstrated endothelial dysfunction in experimental hypertension with monocyte adhesion and transendothelial migration of the adherent cells. The monocytes become macrophages after they enter the intima even though little to no lipid necessarily accumulates in them under these conditions. The combined effects of hyperlipidemia and hypertension in rabbits can lead to marked enhancement of the lesions of atherosclerosis. Thus, the common theme of endothelial dysfunction, inflammation, and a fibroproliferative response recurs in hypertension as well as hyperlipidemia.

Diabetes

The association between diabetes and increased incidence of atherosclerosis is well established. However, there are relatively little data to provide information concerning the basis of the cellular changes that occur in diabetes and the interactions responsible for lesion formation. A number of cellular alterations, including increased glucose metabolism, increased vascular permeability, and altered vascular reactivity, have been suggested as predisposing factors in the arterial injury that could lead to the formation of lesions of atherosclerosis. Diabetic human platelets have been reported to release more growth factor activity after lysis than nondiabetic platelets.[121] Such platelets release not only PDGF but also IGF-I, and injured arteries expressed increased messenger RNA for IGF-I, suggesting a possible autocrine role for this mitogenic and chemotactic factor.[122] Hyperinsulinemia is a common finding in diabetics, and, *in vitro*, insulin and the IGFs augment the proliferative effects of growth factors and stimulate uptake of LDL by smooth muscle cells as well as increase in cholesterol synthesis.

Substances, termed advanced glycosylation endproducts (AGE), accumulate in diabetic tissues, may be toxic, and may lead to the cellular alterations associated with atherogenesis.[123] Aminoguanidine has been observed to prevent the formation of AGE in diabetic rats and thus has permitted experimental demonstration of the potential significance of these substances *in vivo*. Furthermore, monocyte/macrophages contain receptors for AGEs with a Kd of approximately 10^{-7} mol/L, which can be up-regulated by TNF-α or IL-1.[124] Paradoxically, the uptake of AGEs by such stimulated macrophages results in increased release of TNF-α and IL-1 *in vitro*. Furthermore, AGEs are chemotactic for human monocytes *in vitro* and could play a role in atherogenesis as vascular connective tissue matrix will accumulate AGEs with time. The presence of the AGEs in the lesions of atherosclerosis seems well established. Their role in the pathogenesis of the process requires an appropriate animal model of this disease. Unfortunately, there is no animal model yet available that is susceptible to diabetes in which atherosclerosis can also be induced. Thus, additional information will be required from human studies if we are to better understand the relationship between these two disease entities.

Cigarette Smoking

Although the epidemiological association between cigarette smoking, increased incidence of atherosclerosis, and cardiovascular disease is well established, the cellular and molecular events associated with cigarette smoking are essentially unknown. Once again, the absence of an appropriate animal model, as in diabetes, which would permit an analysis of cellular and molecular interactions and changes, has hampered our ability to probe this process.

Free radicals and other mutagenic substances, including oxidized radicals, may increase in the plasma in association with cigarette smoking and thus have been suggested to play a role in atherogenesis. Cigarette smoking also decreases the levels of high-density lipoprotein in some individuals,

which may somehow lead to increased susceptibility to atherosclerosis. Although there have been many speculative suggestions, relatively little hard data exist to explain the statistically demonstrable association.

Immune Injury

Numerous observations suggest that some form of immune injury may be associated with the process of atherogenesis.[125–135] The circumferential lesions of atherosclerosis (Figure 2) that develop in rejected, transplanted hearts and that contain large numbers of monocyte-derived macrophages and T lymphocytes with intense smooth muscle proliferation support this notion.[134] At the same time, as discussed earlier, the lesions of common atherosclerosis are also well endowed with T lymphocytes, often associated with monocyte-derived macrophages.[9] They have been observed in human fatty streaks, as well as in intermediate and advanced lesions.[28,29] The studies of rejected, transplanted hearts demonstrated the presence of human leukocyte antigen-DR (HLA-DR+) endothelial cells with exceedingly high levels of HLA-DR.[127]

All of these lesions contain relatively large numbers of T lymphocytes and macrophages. Many of the T cells bear CD4 markers for the helper-inducer subclass of T cells, whereas others bear the CD8 marker associated with cytolytic T cells. To determine whether or not the T cells in fibrous plaques are innocent bystanders or represent immunologically activated participants, Holm and Hansson asked whether the smooth muscle cells adjacent to the lymphocytes in the lesions express class II histocompatibility antigens that would require the presence of activated T cells. Not only do the smooth muscle cells express major histocompatibility complex-II,[128] but these cells express IL-2 receptors, as well as histochemically detectable interferon-γ.

In contrast, in normal endothelium or endothelium lining the lesions of common atherosclerosis, there are relatively rare HLA-DR+ endothelial cells. However, associated with the antigen-presenting endothelium is a ring of periluminal CD4+ and CD8+ T lymphocytes as well as large numbers of HLA-DR+ macrophages. These observations coupled with a series of *in vitro* studies suggest that interactions between endothelium and CD4+ T cells stimulate the T cells to replicate and increase their secretion of IL-2, leading to a sustained immune response.

Regression of Atherosclerosis

During the past several years, unequivocal evidence has accumulated using quantitative analysis of angiograms, that patients who are aggressively treated to lower their plasma cholesterol and LDL levels, show angiographically demonstrable quantitative regression of their lesions of atherosclerosis. In several studies, Brown et al[136] and Kane et al[137] have observed that marked reductions in plasma cholesterol and LDL levels result in statistically significant increases of their luminal diameter as measured in their angiograms. In examining the series of patients that were treated, some lesions progress, some regress, and some remain unchanged. As a group, however, the patients demonstrated statistically significant association between cholesterol lowering and lesion regression. These data and other observations[138] demonstrate the dynamic character of the lesions of atherosclerosis (as well as the thrombi that provide some of the occlusion as these cannot be differentiated by angioplasty) and suggest that in many patients treated with such aggressive therapy it should be possible to induce lesion regression.

Future Directions

If an excess in the inflammatory and fibroproliferative responses represents the hallmark of atherogenesis, then attempts to optimize these responses, rather than prevent them totally, should represent a logical approach to treatment and/or prevention of atherosclerosis. Many points exist in each of these responses where intervention or prevention might be useful. However, in some cases, total ablation of an inflammatory or reparative response might be harmful.

It would be helpful to determine if there is any selectivity or specialization in the cellular interactions that are related to the different risk factors, or to particular segments of the arterial tree. The demonstration of intercellular networks of communication that are vital to the success of each phase of inflammation and fibroplasia presents opportunities to decrease the level of the response without preventing the response entirely. For example, if several growth factors work in concert, preventing the action of one may be sufficient to reduce the response without ablating it altogether. If infectious agents such as viruses[139] or other agents should be shown to be etiological, this would then point to obvious directions in preventing this inflammatory-fibroproliferative response.

The development of peptidomimetics that compete at numerous levels with the different receptor-

ligand interactions may offer specific opportunities to intervene. This will require determination of the molecular anatomy of the different interactions among the different cytokines and growth regulatory molecules if intervention is to be possible. The opportunity to control these cellular interactions and to optimize the inflammatory and reparative responses represents exciting prospects for future research and therapy.

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